Identifying the Location of Combination Sensitive Neurons in *Hyla cinerea*

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Hypothesis

H1: Combination-sensitive neurons (neurons that response to high and low frequencies auditory stimuli) in *H. cinerea* torus semicircularis are segregated in different nuclei.

Background

In noisy social settings such as a cocktail party, human listeners face the challenge of understanding speech amidst a noisy background[1]. Non-human animals, such as frogs that communicate in large social aggregations, encounter an analogous situation[2]. Males *Hyla cinerea* produce loud complex advertisement calls and these calls combine to form chorus noises[3]. During breeding season, females *H. cinerea* face the challenge of selecting same-species mates among mixed-species breeding aggregations[3].

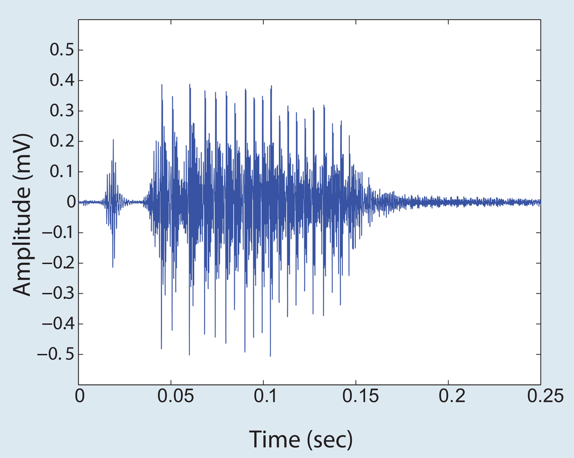


Fig. 1
Waveform of a natural advertisement call of a male *Hyla cinerea*. Males produce calls containing several spectral peaks; with low and high frequency

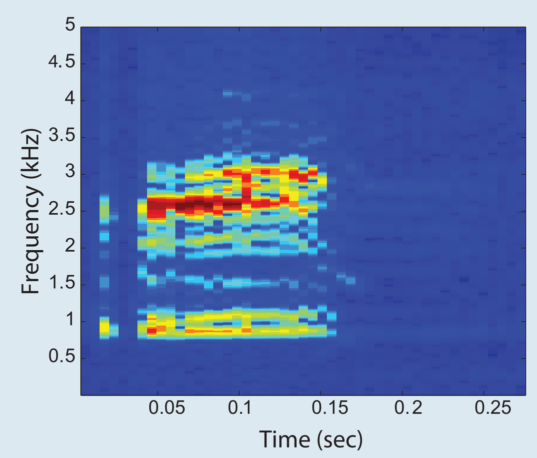


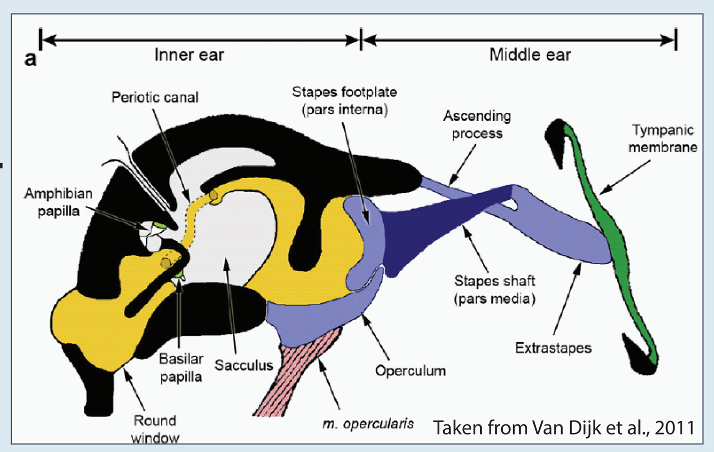
Fig. 2
Spectrogram of males H. cinerea natural call. Red and yellow regions represent significant neural activites while blue regions show low neural activities.

Females *H. cinerea* respond optimally to calls that have low frequency (900-1,100 Hz) and high frequency (2,700-3,300 Hz) presented together[4] (Fig. 1,Fig. 2). The frog's auditory system is unique among other animals because the middle ear has two anatomically distinct end organs: amphibian papilla (AP) and the basilar papilla[5] (BP) (Fig. 3). AP transduced low frequency components while BP transduced high frequency components[5].

Auditory Grouping

Studies suggest that sound elements sharing common features tend to be grouped together into coherent auditory objects[1]. Combination-sensitive auditory neurons in the torus semicircularis (TS) integrate inputs from the AP and BP to produce excitatory or inhibitory responses, depending on the combination of inputs and the classes of neuron[6].

Fig. 3
Diagram of frogs' auditory organs. The amphibian papilla and basilar papilla operate on a tectorial membrane that moves in response to sound vibrations found in no other animal group.



References

(1) **McDermott, J.H.** (2009). Cocktail Party Problem. Current Biology, 18(22), pp.1024-1027. (2) **Bee, M.A.** (2012). Sound source perception in anuran amphibians. Current Opinion in Neurobiology, 22, pp.1-10. (3) **Schwartz, J. J. & Bee, M. A.** (2013). Anuran acoustic signal production in noisy environments. In Brumm, H. (Ed.), Animal communication and noise (pp. 91-124). (4) **Gerhardt, H. C.** (1974). The significance of some spectral features in mating call recognition in green tree frog (Hyla cinerea). Journal of Experimental Biology, 61, pp.229 - 241. (5) **Simmons, D.D., Meenderink, W.F., & Vassilakis, P.N.** (2007). Anatomy, physiology, and function of auditory end-organs in the frog inner ear. In Narins, P. M., Feng, A. S., Fay, R. R., & Popper, A. N (Ed.). Hearing and sound communication in amphibians. New York, NY: Springer. (6) **Fuzessery, Z.M. & Feng, A.S.** (1983). Mating call selectivity in the thalamus and midbrain of the leopard frog (Rana p. pippins): Single and multiunit analyses. Journal of Comparative Physiology, 150, pp.333-344.

Methods

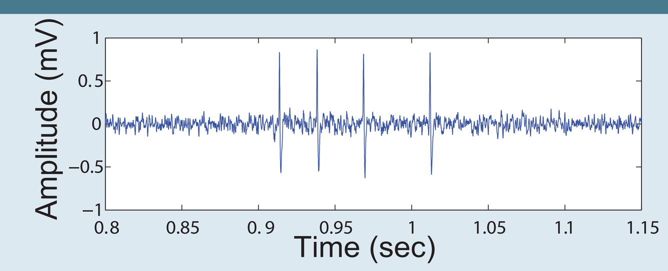


Fig. 4
Oscillogram of single-unit auditory neural response to the stimulus. Spikes threshold are > 0.50 mV in amplitude.

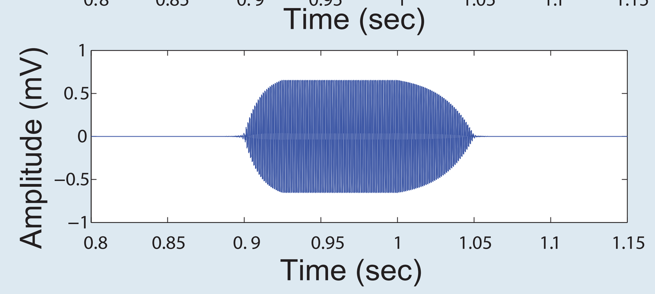


Fig. 5
Oscillogram of males *H. cinerea* synthetic advertisement call that consist of dual frequencies components: 900 Hz and 2,700 Hz.

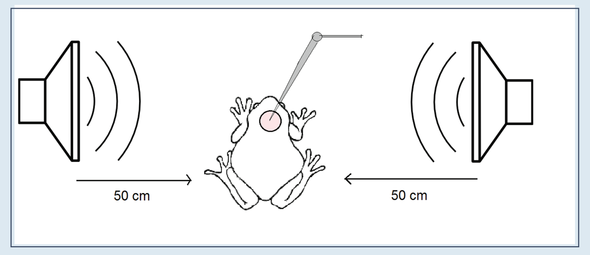


Fig. 6
Diagram of speaker's position relative to the frog's auditory organs.

- Single-unit recordings were obtained from the torus semicircularis using glass microelectrodes.
- Female *H. cinerea* was immobilized with succinylcholine chloride.
- A synthetic advertisement call that consist of two spectral peaks: 900 Hz and 2,700 Hz (Fig. 5) was broadcasted at the intensity of 85 dB. The two spectral peaks are temporally shifted by 0 msec (zero delay), ±12.5 msec, ±25 msec, ±50 msec, ±100 msec and ±150 msec.The auditory neural response was measure as the number of spikes elicited that are > 0.5 mV in amplitude (Fig. 4).
- Stimulus was presented at varying intensity levels across a range of frequencies from 500 Hz to 5000 Hz to produce a frequency tuning curve (FTC).
- Auditory neurons found were traced using horseradish peroxidase (HRP) by injecting the enzyme with a positive current (300 nA) for 30 min.
- Intracardiac perfusion was performed on *H. cinerea* to remove the brain.
- Brain removed from the perfused animal was prepared at 20-25 microns thickness using a cryostat.

Results

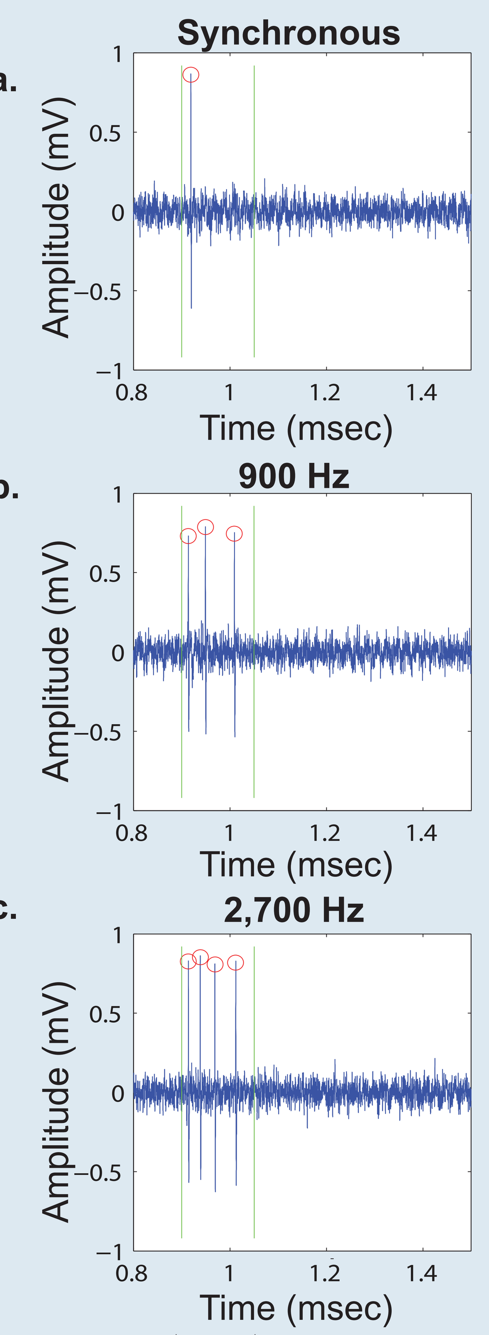


Fig. 7
Raw traces of spike counts in different stimulus conditions. Auditory neural responses of female *H. cinerea* when: (**7a**) stimulus is presented at the same time; (**7b**) when presented alone at low spectral frequency and (**7c**) at high spectral frequency. Each stimulus condition is presented across ten repetitions. For high frequency (2,700 Hz) stimulus, greater spike counts (4) indicate more responses from neuron than other stimuli conditions.

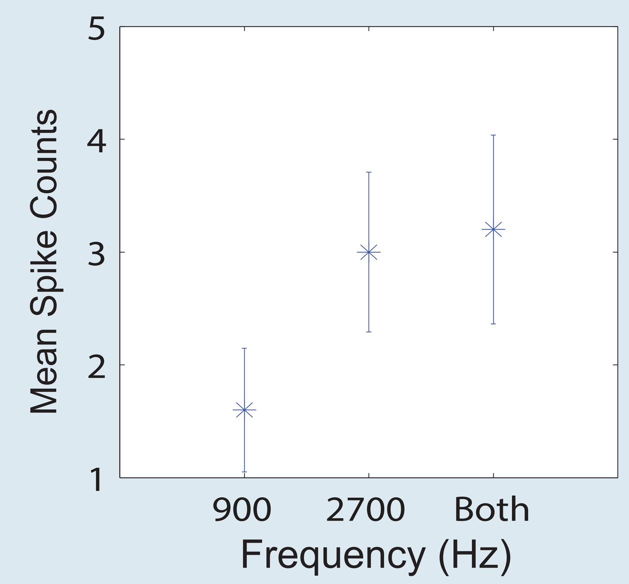


Fig. 8
Summary of analyzed spike rates. Neuron responds to both frequencies when presented alone and when presented together. Responses when both frequencies are presented together are similar to responses when 2,700 Hz was presented alone.

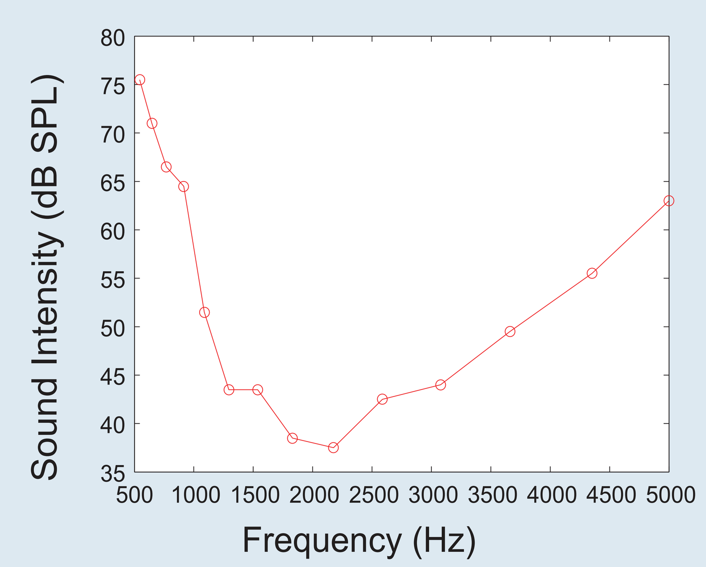


Fig. 9
FTC of neural response. Sound intensity (y-axis) is plotted against the frequency of the stimulus (x-axis). Neuron is most sensitive in the frequency range between 1,700- 2,200 Hz.

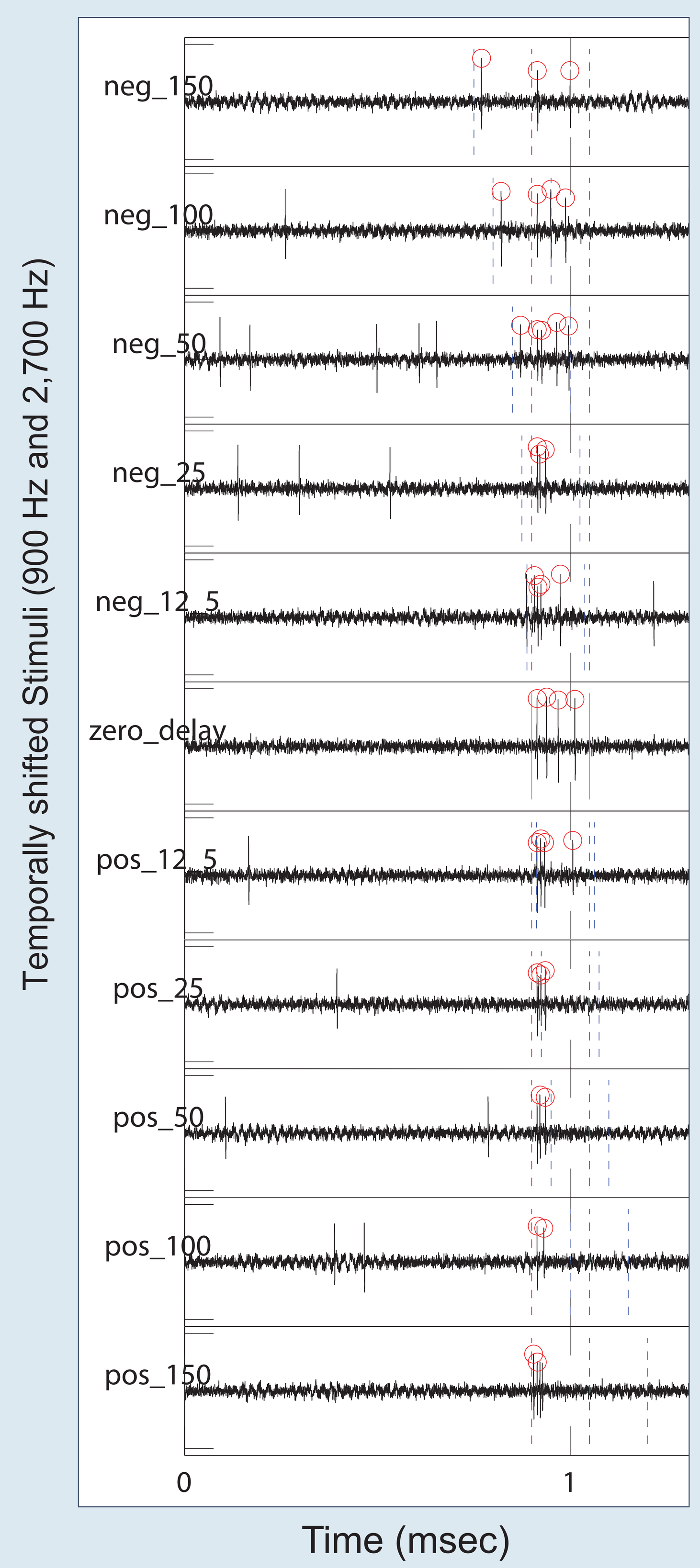


Fig. 10
Raw traces of spike rates for temporally shifted frequencies. Frequency components (900 Hz and 2,700 Hz) are temporally shifted (0, ±12.5, ±25, ±50, ±100 and ±150 msec). Positive indicates 900 Hz component is first presented and negative indicates the alternative.

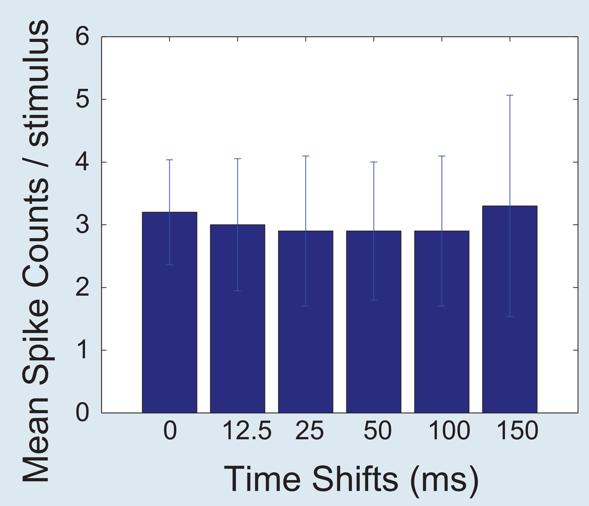


Fig. 11
Summary of mean spike counts and standard deviations of raw traces for temporally shifted frequencies. The mean spike counts is between 3-4 across all time shifts.

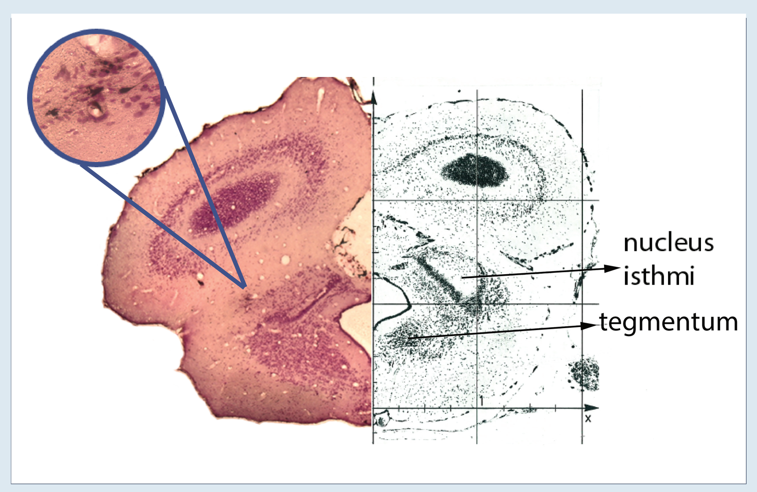


Fig. 12
Single-unit recordings in the TS. Blue circle: HRP labeled recording site.

- The auditory neuron found responds to all stimulus conditions; the response to 2,700 Hz is similar to the response of the synchronous stimulus (Fig. 7, Fig. 8).
- The single-unit is a 2,700 Hz multi-tone responders (MTRs) because it responds best to stimuli at 2,700 Hz across ten repetitions (Fig. 9). The FTC matches with the summary of analyzed spike rates.
- 2,700 Hz MTRs is not responsible for auditory grouping grouping because there is no correlations between the degree of time shifts and the mean spike counts (Fig. 10, Fig. 11).
- Traces of MTRs are found within the TS (Fig. 12).

Conclusions

- FTC and spike rates summary conclude that auditory unit found is a 2,700 Hz MTRs.
- Both excitatory inputs from individual frequency stimuli (900 Hz and 2,700 Hz) does not sum up to a greater response.
- 2,700 MTRs unit found is not responsible for auditory grouping.
- 2,700 MTRs found is within the torus semicircularis of the frog's (*H. cinerea*) brain.